

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claim 1 (withdrawn): A method for the production of glycerol from a recombinant organism comprising:

- (i) transforming a suitable host cell with an expression cassette comprising either one or both of
 - (a) a gene encoding a protein having glycerol-3-phosphate dehydrogenase activity, and
 - (b) a gene encoding a protein having glycerol-3-phosphate phosphatase activity,the suitable host cell having a disruption in either one or both of
 - (a) an endogenous gene encoding a polypeptide having glycerol kinase activity, and
 - (b) an endogenous gene encoding a polypeptide having glycerol dehydrogenase activity,wherein the disruption prevents the expression of active gene product;
- (ii) culturing the transformed host cell of (i) in the presence of at least one carbon source selected from the group consisting of monosaccharides, oligosaccharides, polysaccharides, and single-carbon substrates, whereby glycerol is produced; and
- (iii) optionally recovering the glycerol produced in (ii).

Claim 2 (withdrawn): The method of Claim 1 wherein the expression cassette comprises a gene encoding a glycerol-3-phosphate dehydrogenase enzyme.

Claim 3 (withdrawn): The method of Claim 1 wherein the expression cassette comprises a gene encoding a glycerol-3-phosphate phosphatase enzyme.

Claim 4 (withdrawn): The method of Claim 1 wherein the expression cassette comprises genes encoding a glycerol-3-phosphate phosphatase enzyme and a glycerol-3-phosphate dehydrogenase enzyme.

Claim 5 (withdrawn): The method of Claim 1 wherein the host cell contains a disruption in a gene encoding an endogenous glycerol kinase enzyme wherein the disruption prevents the expression of active gene product.

Claim 6 (withdrawn): The method of Claim 1 wherein the host cell contains a disruption in a gene encoding an endogenous glycerol dehydrogenase enzyme wherein the disruption prevents the expression of active gene product.

Claim 7 (withdrawn): The method of Claim 1 wherein the host cell contains a) a disruption in a gene encoding an endogenous glycerol kinase enzyme and b) a disruption in a gene encoding an endogenous glycerol dehydrogenase enzyme, wherein the disruptions in the respective genes prevent the expression of active gene product from either gene.

Claim 8 (withdrawn): The method of Claim 1 wherein the suitable host cell is selected from the group consisting of bacteria, yeast, and filamentous fungi.

Claim 9 (withdrawn): The method of Claim 8 wherein the suitable host cell is selected from the group consisting of *Citrobacter*, *Enterobacter*, *Clostridium*, *Klebsiella*, *Aerobacter*, *Lactobacillus*, *Aspergillus*, *Saccharomyces*, *Schizosaccharomyces*, *Zygosaccharomyces*, *Pichia*, *Kluyveromyces*, *Candida*, *Hansenula*, *Debaryomyces*, *Mucor*, *Torulopsis*, *Methylbacter*, *Escherichia*, *Salmonella*, *Bacillus*, *Streptomyces*, and *Pseudomonas*.

Claim 10 (withdrawn): The method of Claim 9 wherein the suitable host cell is *E. coli* or *Saccharomyces* sp.

Claim 11 (withdrawn): The method of Claim 1 wherein the carbon source is glucose.

Claim 12 (withdrawn): The method of Claim 1 wherein the protein having glycerol-3-phosphate dehydrogenase activity corresponds to amino acid sequences selected from the group consisting of SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12 and wherein the amino acid sequences encompasses amino acid substitutions, deletions or insertions that do not alter the functional properties of the enzyme.

Claim 13 (withdrawn): The method of Claim 1 wherein the protein having glycerol-3-phosphatase activity corresponds to the amino acid sequences selected from the group consisting of SEQ ID NO:13 and SEQ ID NO:14, and wherein the amino acid sequences may encompass amino acid substitutions, deletions or additions that do not alter the function of the enzyme.

Claim 14 (currently amended): A transformed host cell comprising:

- (a) a an exogenous gene encoding a protein having a glycerol-3-phosphate dehydrogenase activity;
- (b) a an exogenous gene encoding a protein having glycerol-3-phosphate phosphatase activity;
- (c) a disruption in a gene encoding an endogenous glycerol kinase, wherein the disruption prevents the expression of the active gene product; and [[;]]
- (d) a disruption in a gene encoding an endogenous glycerol dehydrogenase [[;]] , wherein the disruption prevents the expression of the active gene product.

~~wherein the disruptions in the genes of (c) and (d) prevent the expression of active gene product, and wherein the host cell converts at least one carbon source selected from the group consisting of monosaccharides, oligosaccharides, polysaccharides, and single carbon substrates to glycerol.~~

Claim 15 (currently amended): A transformed host cell comprising:

- (a) a an exogenous gene encoding a protein having a glycerol-3-phosphate dehydrogenase activity;
- (b) a an exogenous gene encoding a protein having glycerol-3-phosphate phosphatase activity; and
- (c) disruption in a gene encoding an endogenous glycerol dehydrogenase [[;]], wherein the disruption prevents the expression of the active gene product.

~~wherein the disruption in the gene of (c) prevents the expression of active gene product, and wherein the host cell converts at least one carbon source selected from the group consisting of monosaccharides, oligosaccharides, polysaccharides, and single carbon substrates to glycerol.~~

Claim 16 (currently amended): A transformed host cell comprising:

- (a) a an exogenous gene encoding a protein having a glycerol-3-phosphate dehydrogenase activity;
- (b) a an exogenous gene encoding a protein having glycerol-3-phosphate phosphatase activity; and
- (c) a disruption in a gene encoding an endogenous glycerol kinase, wherein the disruption prevents the expression of the active gene product.

~~wherein the disruption in the gene of (c) prevents the expression of active gene product, and wherein the host cell converts at least one carbon source selected from the group consisting of monosaccharides, oligosaccharides, polysaccharides, and single carbon substrates to glycerol.~~

Claim 17 (withdrawn): A method for the production of 1,3-propanediol from a recombinant organism comprising:

- (i) transforming a suitable host cell with an expression cassette comprising either one or both of
 - (a) a gene encoding a protein having glycerol-3-phosphate dehydrogenase activity, and

- (b) a gene encoding a protein having glycerol-3-phosphate phosphatase activity,
the suitable host cell having at least one gene encoding a protein having a dehydratase activity and having a disruption in either one or both of:
 - (a) an endogenous gene encoding a polypeptide having glycerol kinase activity, and
 - (b) an endogenous gene encoding a polypeptide having glycerol dehydrogenase activity,
wherein the disruption in the genes of (a) or (b) prevents the expression of active gene product;
- (ii) culturing the transformed host cell of (i) in the presence of at least one carbon source selected from the group consisting of monosaccharides, oligosaccharides, polysaccharides, and single-carbon substrates whereby 1,3-propanediol is produced; and
- (iii) recovering the 1,3-propanediol produced in (ii).

Claim 18 (withdrawn): The method of Claim 17 wherein the protein having a dehydratase activity is selected from the group consisting of glycerol dehydratase enzyme and a diol dehydratase enzyme.

Claim 19 (withdrawn): The method of Claim 18 wherein the glycerol dehydratase enzyme is encoded by a gene, the gene isolated from a microorganism, the microorganism selected from the group consisting of *Klebsiella*, *Lactobacillus*, *Enterobacter*, *Citrobacter*, *Pelobacter*, *Illyobacter*, and *Clostridium*.

Claim 20 (withdrawn): The method of Claim 18 wherein the diol dehydratase enzyme is encoded by a gene, the gene isolated from a microorganism, the microorganism selected from the group consisting of *Klebsiella* and *Salmonella*.

Claim 21 (withdrawn): A method for the production of glycerol from a recombinant organism comprising:

- (i) transforming a suitable host cell with an expression cassette comprising either one or both of
 - (a) a gene encoding a glycerol-3-phosphate dehydrogenase enzyme selected from the group of enzymes consisting of sn-glycerol-3-phosphate:NAD⁺2-oxidoreductase and sn-glycerol-3-phosphate:NADP⁺2-oxidoreductase;
 - (b) a gene encoding a glycerol-3-phosphate phosphatase enzyme;
- (ii) culturing the transformed host cell of (i) in the presence of at least one carbon source selected from the group consisting of monosaccharides, oligosaccharides, polysaccharides, and single-carbon substrates, whereby glycerol is produced; and
- (iii) recovering the glycerol produced in (ii).

Claim 22 (withdrawn): The method of Claim 21 wherein the gene encoding a protein having glycerol-3-phosphate phosphatase activity is obtained from ATCC deposit 98187 and the gene encoding a protein having glycerol-3-phosphate dehydrogenase activity is obtained from ATCC deposit 98248.

Claim 23 (previously presented): *Escherichia coli* pAH21/DH5 α designated ATCC 98187.

Claim 24 (previously presented): *Escherichia coli* pDAR1A/AA200, designated ATCC 98248.

Claim 25 (previously presented): *Escherichia coli* RJF 10m, designated ATCC 98597.

Claim 26 (previously presented): *Escherichia coli* mSP33.6, designated ATCC 98598.